

AMENDMENTS

To the claims:

Please amend the claims as indicated hereafter.

1. (Currently Amended) A method of isolating islets from a pancreas, comprising the steps of:

controlling one or more process variables of an islet processing solution with a process controller, wherein one of the one or more process variables
~~wherein a process variable describing the chemical character of the islet processing solution~~ is the process temperature (T);
controlling the process temperature (T) of the islet processing solution between 4.0 and 44.0 degrees Celsius; and the process variable is controlled via a setpoint and the process temperature setpoint is between 4.0 degrees Celsius and 44.0 degrees Celsius
and separating one or more islets from the pancreas.

2. (Currently Amended) The method of claim 1, wherein the process controller is a PID (proportional, integral, derivative) controller ~~and the process temperature setpoint is between 4.0 degrees Celsius and 44.0 degrees Celsius.~~

3. (Currently Amended) The method of claim 1, wherein the process controller is a microprocessor temperature controller ~~and the process temperature setpoint is between 4.0 degrees Celsius and 44.0 degrees Celsius.~~

4. (Currently Amended) The method of claim 1, wherein the process controller is a microprocessor controller ~~and the process temperature setpoint is between 4.0 degrees Celsius and 44.0 degrees Celsius.~~

5. (Currently Amended) The method of claim 1, wherein the process controller is a microprocessor computer ~~and the process temperature setpoint is between 4.0 degrees Celsius and 44.0 degrees Celsius.~~

6. (Currently Amended) The method of claim 1, wherein the process controller is a variable resistance transformer ~~and the process temperature setpoint is between 4.0 degrees Celsius and 44.0 degrees Celsius.~~

7. (Currently Amended) The method of claim 1, wherein the process temperature is generated adjusted by an electrical resistance element in thermal communication with the islet processing solution ~~and the process temperature setpoint is between 4.0 degrees Celsius and 44.0 degrees Celsius.~~

8. (Currently Amended) The method of claim 1, wherein the process temperature is ~~generated~~ adjusted by steam placed in thermal communication with the islet processing solution ~~and the process temperature setpoint is between 4.0 degrees Celsius and 44.0 degrees Celsius.~~

9. (Currently Amended) The method of claim 1, wherein the process temperature is ~~generated~~ adjusted by a recirculating fluid bath in thermal communication with the islet processing solution ~~and the process temperature setpoint is between 4.0 degrees Celsius and 44.0 degrees Celsius.~~

10. (Currently Amended) The method of claim 1, wherein the process temperature is ~~generated~~ adjusted by the temperature of the ambient surrounding in thermal communication with the islet processing solution ~~and the process temperature setpoint is between 4.0 degrees Celsius and 44.0 degrees Celsius.~~

11. (Currently Amended) The method of claim 1, wherein the process variable is the process percent hydrogen ion (pH) concentration and the ~~process controller is~~ pH is controlled by a microprocessor pH controller ~~and the process percent hydrogen concentration setpoint is~~ between pH 6.00 and pH 8.00.

12. (Currently Amended) The method of claim 1, wherein the process variable is the process percent hydrogen ion (pH) concentration and the ~~process controller is~~ pH is controlled by a microprocessor controller ~~and the process percent hydrogen concentration setpoint is~~ between pH 6.00 and pH 8.00.

13. (Currently Amended) The method of claim 1, wherein the process variable is the process percent hydrogen ion (pH) concentration and the ~~process controller is~~ pH is controlled by a microprocessor computer ~~and the process percent hydrogen concentration setpoint is~~ between pH 6.00 and pH 8.00.

14. (Currently Amended) The method of claim 1, wherein the process variable is the process percent hydrogen ion (pH) concentration and the ~~process percent hydrogen concentration~~ pH is controlled by the addition of an acid or base to the islet processing solution ~~process solution thereby buffering the process percent hydrogen concentration in the process solution between pH 6.00 and pH 8.00.~~

15. (Currently Amended) The method of claim 1, wherein the process variable is the process flowrate (F) and the ~~process controller is~~ flowrate is controlled by a microprocessor flow controller ~~and the process flowrate setpoint is~~ between 10.0 milliliters per minute (10.0 ml/min) and 4000.0 milliliters per minute (4000.0 ml/min).

16. (Currently Amended) The method of claim 1, wherein the process variable is the process flowrate (F) and the ~~process controller is~~ flowrate is controlled by a microprocessor controller ~~and the process flowrate setpoint is~~ between 10.0 milliliters per minute (10.0 ml/min) and 4000.0 milliliters per minute (4000.0 ml/min).

17. (Currently Amended) The method of claim 1, wherein the process variable is the process flowrate (F) and the ~~process controller is~~ flowrate is controlled by a microprocessor computer ~~and the process flowrate setpoint is~~ between 10.0 milliliters per minute (10.0 ml/min) and 4000.0 milliliters per minute (4000.0 ml/min).

18. (Currently Amended) The method of claim 1, wherein the process variable is the process dissolved oxygen (DO) concentration and the ~~process controller is~~ DO concentration is controlled by a microprocessor DO controller ~~and the process dissolved oxygen concentration setpoint is~~ between 0.000000001 milligrams per milliliter (0.000000001mg/ml) DO and ~~2.0~~ 10.0 milligrams per milliliter (~~2.0~~ 10.0 mg/ml) DO.

19. (Currently Amended) The method of claim 1, wherein the process variable is the process dissolved oxygen (DO) concentration and the ~~process controller is~~ DO concentration is controlled by a microprocessor controller ~~and the process dissolved oxygen concentration setpoint is~~ between 0.000000001 milligrams per milliliter (0.000000001mg/ml) DO and ~~2.0~~ 10.0 milligrams per milliliter (~~2.0~~ 10.0 mg/ml) DO.

20. (Currently Amended) The method of claim 1, wherein the process variable is the process dissolved oxygen (DO) concentration and the ~~process controller is DO~~ concentration is controlled by a microprocessor computer ~~and the process dissolved oxygen concentration setpoint is~~ between 0.000000001 milligrams per milliliter (0.000000001mg/ml) DO and ~~2.0~~ 10.0 milligrams per milliliter (~~2.0~~ 10.0 mg/ml) DO.

21. (Currently Amended) The method of claim 1, wherein the process variable is the process dissolved oxygen (DO) concentration and the ~~process dissolved oxygen DO~~ concentration is controlled by sparging the islet processing solution ~~process solution~~ with an inert gas chosen from either helium, ~~or~~ neon, ~~or~~ argon, ~~or~~ krypton, or xenon ~~thereby displacing dissolved oxygen from the process solution.~~

22. (Currently Amended) The method of claim 1, wherein the process variable is the process dissolved nitric oxide (NO) concentration and the ~~process controller is NO concentration~~ is controlled by a microprocessor NO controller ~~and the process dissolved nitric oxide concentration setpoint is~~ between 0.00000000000001 moles per liter (0.01 picomoles/liter) NO and ~~0.01~~ 1.0 mole per liter (~~0.01~~ 1.0 mol/liter) NO.

23. (Currently Amended) The method of claim 1, wherein the process variable is the process dissolved nitric oxide (NO) concentration and the ~~process controller is NO~~ concentration is controlled by a microprocessor controller ~~and the process dissolved nitric oxide concentration setpoint is~~ between 0.00000000000001 moles per liter (0.01 picomoles/liter) NO and ~~0.01~~ 1.0 mole per liter (~~0.01~~ 1.0 mol/liter) NO.

24. (Currently Amended) The method of claim 1, wherein the process variable is the process dissolved nitric oxide (NO) concentration and the ~~process controller is NO~~ concentration is controlled by a microprocessor computer ~~and the process dissolved nitric oxide concentration setpoint is~~ between 0.00000000000001 moles per liter (0.01 picomoles/liter) NO and ~~0.01~~ 1.0 mole per liter (~~0.01~~ 1.0 mol/liter) NO.

25. (Currently Amended) The method of claim 1, wherein the process variable is the process dissolved nitric oxide (NO) concentration and the ~~process dissolved nitric oxide~~ NO concentration is controlled by sparging the islet processing solution ~~process solution~~ with an inert gas chosen from either helium, ~~or~~ neon, ~~or~~ argon, ~~or~~ krypton, or xenon ~~displacing dissolved oxygen from the process solution thereby inhibiting nitric oxide in the process solution.~~

26. (Currently Amended) The method of claim 1, wherein the process variable is the process endotoxin (E) concentration and the ~~process controller is~~ endotoxin concentration ~~is controlled by a microprocessor E controller and the process endotoxin concentration setpoint is~~ between 0.000000001 endotoxin units (EU) per milligram (1.0 nanoEU/mg) and 100.0 endotoxin units per milligram (100.0 EU/mg).

27. (Currently Amended) The method of claim 1, wherein the process variable is the process endotoxin (E) concentration and the ~~process controller is~~ endotoxin concentration ~~is controlled by a microprocessor controller and the process endotoxin concentration setpoint is~~ between 0.000000001 endotoxin units (EU) per milligram (1.0 nanoEU/mg) and 100.0 endotoxin units per milligram (100.0 EU/mg).

28. (Currently Amended) The method of claim 1, wherein the process variable is the process endotoxin (E) concentration and the ~~process controller is~~ endotoxin concentration ~~is controlled by a microprocessor computer and the process endotoxin concentration setpoint is~~ between 0.000000001 endotoxin units (EU) per milligram (1.0 nanoEU/mg) and 100.0 endotoxin units per milligram (100.0 EU/mg).

29. (Currently Amended) The method of claim 1, wherein the process variable is the process endotoxin (E) concentration and the ~~process~~ endotoxin concentration is controlled by the addition of endotoxin neutralizing protein (ENP) to the islet processing solution ~~process solution thereby neutralizing endotoxin in the process solution.~~

30. (Currently Amended) The method of claim 1, wherein the process variable is the process endotoxin neutralizing protein (ENP) concentration and the ~~process controller is~~ ENP concentration is controlled by a microprocessor ENP controller ~~and the process endotoxin neutralizing protein concentration setpoint is~~ between 0.000000000000001 moles per liter (0.01 picomoles/liter) ENP and ~~0.01~~ 1.0 moles per liter (~~0.01~~ 1.0 mol/liter) ENP.

31. (Currently Amended) The method of claim 1, wherein the process variable is the process proteolytic enzyme [PE] activity (measured by the ~~process~~ metalloendoproteinase [collagenase] concentration) and the ~~process~~ proteolytic enzyme activity ~~of collagenase classes I and II~~ is controlled by the addition of one or more antibiotics to the islet processing solution ~~chosen from~~ tetracycline, ~~or~~ minocycline, or doxycycline ~~to the process solution thereby neutralizing metalloendoproteinase (collagenase collagenase) in the process solution.~~

32. (Currently Amended) The method of claim 1, wherein the process variable is the process proteolytic enzyme [PE] activity (measured by the ~~process~~ metalloendoproteinase [collagenase] concentration) and the ~~process~~ proteolytic enzyme activity of ~~collagenase classes I and II~~ is controlled by the addition of one or more chelators of divalent cations to the islet processing solution chosen from ~~either~~ citrate, ~~or~~ EDTA, or EGTA ~~to the process solution~~ ~~thereby neutralizing metalloendoproteinase (collagenase) in the process solution.~~

33. (Currently Amended) The method of claim 1, wherein the process variable is the process proteolytic enzyme [PE] activity (measured by the ~~process~~ metalloendoproteinase [collagenase] concentration) and the ~~process~~ proteolytic enzyme activity of ~~collagenase classes I and II~~ is controlled by the addition of one or more amino acids to the islet processing solution chosen from ~~either~~ cysteine or cystine ~~to the process solution~~ ~~thereby neutralizing metalloendoproteinase (collagenase) in the process solution.~~

34. (Currently Amended) The method of claim 1, wherein the process variable is the process proteolytic enzyme [PE] activity and the ~~process controller is~~ proteolytic enzyme is ~~controlled by a microprocessor proteolytic enzyme neutralization (PEN) controller and the~~ (process proteolytic enzyme [PE] activity measured by the metalloendoproteinase [collagenase] concentration) ~~process metalloendoproteinase (collagenase) setpoint is~~ between 0.00000000000001 moles per liter (0.01 picomoles/liter) and ~~0.01~~ 1.0 moles per liter (~~0.01~~ 1.0 mol/liter).

36. (Currently Amended) The method of claim 1, wherein the process variable is the process antibiotic (A) concentration and the ~~process controller is~~ antibiotic concentration is ~~controlled by a microprocessor proteolytic enzyme neutralization (PEN) controller and the~~ process antibiotic concentration setpoint is between 0.000000000000001 moles per liter (0.01 picomoles/liter) A and ~~0.01~~ 1.0 mole per liter (~~0.01~~ 1.0 mol/liter) A.

37. (Currently Amended) The method of claim 1, wherein the process variable is the process nitric oxide synthase (NOS) concentration and the ~~process~~ nitric oxide synthase concentration is controlled by the addition of one or more derivatives of L-arginine to the islet processing solution chosen from ~~either~~ aminoguanidine, ~~or~~ N, N'-diaminoguanidine, ~~or~~ methylguanidine, or 1, 1-dimethylguanidine ~~to the process solution thereby inhibiting nitric oxide synthase in the process solution.~~

38. (Currently Amended) The method of claim 1, wherein the process variable is the process nitric oxide synthase (NOS) concentration and the ~~process~~ nitric oxide synthase concentration is controlled by the addition of 2,4-diamino-6-hydroxy-pyrimidine to the islet processing solution ~~process solution thereby inhibiting nitric oxide synthase in the process solution.~~

39. (Currently Amended) The method of claim 1, wherein the process variable is the islet processing solution ~~process solution~~ pressure (P) and the ~~process solution~~ pressure ~~setpoint~~ is between ~~5.0 pounds~~ 1.0 pound per square inch gauge (psig) pressure and 150.0 pounds per square inch gauge (psig) pressure.

40. (Currently Amended) The method of claim 1, wherein the process variable is the process carbon ~~dioxide (CO₂)~~ monoxide (CO) concentration and the ~~process dissolved carbon dioxide~~ monoxide concentration is controlled by sparging the islet processing solution ~~process solution~~ with carbon monoxide ~~an inert gas either helium, or neon, or argon, or krypton, or xenon thereby displacing dissolved carbon dioxide from the process solution.~~

41. (Currently Amended) The method of claim 1, wherein the pancreas is a human pancreas.

42. (Currently Amended) The method of claim 1, wherein the pancreas is a transgenic porcine pancreas.

43. (Currently Amended) The method of claim 1, wherein the pancreas is a non-transgenic porcine pancreas.

44. (Currently Amended) The method of claim 1, wherein the pancreas is a transgenic mammalian pancreas.

45. (Currently Amended) The method of claim 1, wherein the pancreas is a non-transgenic mammalian pancreas.

46. (Currently Amended) The method of claim 1, wherein the pancreas is a transgenic fish pancreas.

47-60. (Canceled)

61. (New) A method of isolating islets from a pancreas, comprising the steps of:
controlling one or more process control variables of an islet processing solution in
a predetermined manner, the one or more process control variables chosen
from the following: temperature, pH, flowrate, dissolved oxygen
concentration, dissolved nitric oxide concentration, nitric oxide synthase
concentration, endotoxin concentration, endotoxin neutralizing protein
concentration, antibiotic concentration, amino acid concentration, dextran
concentration, heparin concentration, or proteolytic enzyme activity; and
separating one or more islets from a pancreas while the one or more process
variables is controlled.
62. (New) The method claim 61, wherein a process variable is the process proteolytic
enzyme [PE] activity and the proteolytic enzyme activity is controlled by the addition of
antibiotics to the islet processing solution.
63. (New) The method claim 61, wherein a process variable is the process proteolytic
enzyme [PE] activity and the proteolytic enzyme activity is controlled by the addition of
chelators of divalent cations to the islet processing solution.

64. (New) The method of claim 61, wherein a process variable is the process proteolytic enzyme [PE] activity and the proteolytic enzyme activity is controlled by the addition of amino acids to the islet processing solution.

65. (New) The method of claim 61, wherein a process variable is the process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by the addition one or more of derivatives of L-arginine to the islet processing solution chosen from aminoguanidine, N, N'-diaminoguanidine, methylguanidine, or 1, 1-dimethylguanidine.

66. (New) The method of claim 61, wherein a process variable is the process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by the addition of 2,4-diamino-6-hydroxy-pyrimidine to the islet processing solution.

67. (New) The method of claim 61, wherein a process variable is the process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by the addition of amino acids to the islet processing solution.

68. (New) The method of claim 61, wherein a process variable is the process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by the addition of one or more of the following to the islet processing solution chosen from dextran or heparin.

69. (New) The method of claim 61, wherein a process variable is the process dissolved nitric oxide (NO) concentration and the NO concentration is controlled or inhibited by the addition one or more antibiotics to the islet processing solution chosen from tetracycline, minocycline, or doxycycline.

70. (New) The method of claim 61, wherein a process variable is the process nitric oxide synthase (NOS) concentration and the NOS concentration is controlled or inhibited by the addition of one or more antibiotics to the islet processing solution chosen from tetracycline, minocycline, or doxycycline.

71. (New) The method of claim 61, wherein apoptosis of beta cells is inhibited during and after islet isolation by controlling the endotoxin concentration in the islet processing solution.

72. (New) The method of claim 61, wherein apoptosis of beta cells is inhibited during and after islet isolation by controlling the nitric oxide concentration in the islet processing solution.